

What is claimed is:

1. A kit for determining a concentration of a vitamin D component comprising:

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a releasing composition; and

a detecting composition,

10 the releasing composition comprises an aqueous base component and facilitates in releasing the vitamin D component from a vitamin D component binding-protein, the detecting composition facilitates in determining the concentration of the vitamin D component.

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2. A kit of claim 1 being useful for determining the concentration of the vitamin D component present in a mammal fluid.

20 3. A kit of claim 1 wherein the mammal fluid is selected from the group consisting of milk, whole blood, serum, plasma and mixtures thereof.

25 4. A kit of claim 1 wherein the mammal fluid comprises a human serum.

5. A kit of claim 1 wherein the vitamin D component is selected from the group consisting of a metabolite of vitamin D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>.

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6. A kit of claim 1 wherein the vitamin D component comprises a 25-OH-D.

35 7. A kit of claim 1 wherein the vitamin D component comprises a 1, 25-(OH)<sub>2</sub>-D.

8. A kit of claim 1 wherein the aqueous base component comprises NaOH.

9. A kit of claim 1 wherein the aqueous base component  
5 comprises KOH.

10. A kit of claim 1 wherein the releasing composition comprises about 0.1 to about 1.0 M of the aqueous base component.

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11. A kit of claim 1 wherein the releasing composition comprises about 0.35 to about 0.5 M of the aqueous base component, wherein the aqueous base component is NaOH.

12. A kit of claim 1 wherein the releasing composition is substantially free of an organic solvent.

13. A kit of claim 1 wherein the releasing composition further comprises a cyclo-oligomer component.

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14. A kit of claim 13 wherein the cyclo-oligomer component comprises a cyclodextrin.

15. A kit of claim 13 wherein the cyclo-oligomer  
25 component is selected from the group consisting of alpha-cyclodextrin and beta-randomly methylated cyclodextrin.

16. A kit of claim 13 wherein the releasing composition comprises about 0.01 to about 5% of the cyclo-oligomer  
30 component.

17. A kit of claim 13 wherein the releasing component comprises about 2% of the cyclo-oligomer component, wherein the cyclo-oligomer component is an alpha-  
35 cyclodextrin.

18. A kit of claim 13 wherein the releasing component comprises about 0.05% of the cyclo-oligomer component, wherein the cyclo-oligomer component is a beta-randomly methylated cyclodextrin.

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19. A kit of claim 1 wherein the releasing component further comprises about 0.5 to about 5% of a metal salicylate, including sodium salicylate.

10 20. A kit of claim 1 wherein the releasing component further comprises about 0.01 to about 0.1% of a surfactant.

15 21. A kit of claim 20 wherein the surfactant is selected from the group consisting of tween-20 and triton X-100.

22. A kit of claim 1 wherein the releasing composition forms a homogeneous mixture with a mammal fluid.

20 23. A kit of claim 1 wherein the releasing composition comprises

about 0.1 to about 1.0 M of an aqueous base component;

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about 0.01 to about 5% of a cyclo-oligomer component; and

about 0.01 to about 5% of a metal salicylate.

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24. A kit of claim 23 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin and the metal salicylate is sodium salicylate.

35 25. A kit of claim 1 wherein the detecting composition comprises a host component and a partner component,

wherein the host component binds to the partner component to form a partner/host complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

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26. A kit of claim 25 wherein the host component comprises an antibody, portions thereof, or mixtures thereof.

10 27. A kit of claim 25 wherein the host component is labeled with a chemiluminescent label, a fluorescent label or a radio-active label.

15 28. A kit of claim 25 wherein the host component is an antibody labeled with acridinium.

20 29. A kit of claim 25 wherein the partner component comprises a vitamin D component linked to a separator component, the separator component is a solid phase or an antibody.

30. A kit of claim 29 wherein the separator component comprises a magnetic particle.

25 31. A kit of claim 29 wherein the partner component comprises a vitamin D component linked to a magnetic particle.

30 32. A kit of claim 31 wherein the partner component competes with the vitamin D component to bind to the host component.

33. A kit of claim 32 wherein the host component is an antibody labeled with acridinium.

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34. A kit of claim 32 wherein the partner component

binds to the host component through at least one intermediate binding component.

35. A kit of claim 34 wherein the intermediate component  
5 is labeled.

36. A kit of claim 34 wherein the intermediate component is labeled and the host component is not labeled.

10 37. A kit of claim 34 wherein at least one intermediate binding component comprise a vitamin D binding-protein.

38. A kit of claim 25 wherein the partner component competes with a vitamin D component to form a complex  
15 with the host component, the partner component comprises a vitamin D component linked to a magnetic particle, the partner component binds to the host component through a vitamin D binding-protein, the host component comprises an antibody labeled with acridinium.

20 39. A kit of claim 38 wherein the concentration of the complex is inversely proportional to the concentration of the vitamin D component.

25 40. A kit of claim 1 wherein the concentration of the vitamin D component is determined with a higher precision than that of an assay kit relying on an organic solvent, to release the vitamin D component from the binding-protein.

30 41. A kit of claim 1 wherein the releasing composition forms a homogeneous mixture with a body fluid containing the vitamin D component.

35 42. A kit for determining a concentration of a vitamin D component comprising:

a releasing composition comprising about 0.1 to about 1.0 M of NaOH or KOH, 0 to about 5% of a cyclodextrin, 0 to about 5% of salicylate and 0 to about  
5 0.1% of a surfactant,

a detecting composition comprising an antibody labeled with acridinium and a partner component, wherein the partner component competes with a vitamin D component  
10 to form a complex with the antibody, the partner component comprises a vitamin D component linked to a magnetic particle, the partner component binds to the antibody through a vitamin D binding-protein.

15 43. A kit of claim 42 wherein the releasing composition comprises about 0.35 to about 0.5 M of NaOH, about 2% of alpha-cyclodextrin and about 2% of salicylate, the releasing composition being substantially free of an organic solvent.

20 44. A releasing composition comprising an aqueous base component, wherein the releasing composition facilitates in releasing a vitamin D component from a binding-protein, the releasing composition being useful in  
25 determining the concentration of a vitamin D component.

45. A releasing composition of claim 44 wherein the aqueous base component comprises NaOH.

30 46. A releasing composition of claim 44 wherein the aqueous base component comprises KOH.

47. A releasing composition of claim 44 comprising about 0.1 to about 1.0 M of the aqueous base component.

35 48. A releasing composition of claim 44 comprising about

0.35 to about 0.5 M of the aqueous base component,  
wherein the aqueous base component is NaOH.

49. A releasing composition of claim 44 being  
5 substantially free of an organic solvent.

50. A releasing composition of claim 44 further  
comprising a cyclo-oligomer component.

10 51. A releasing composition of claim 50 wherein the  
cyclo-oligomer component comprises a cyclodextrin.

52. A releasing composition of claim 50 wherein the  
cyclo-oligomer component is selected from the group  
15 consisting of alpha-cyclodextrin and beta-randomly  
methylated cyclodextrin.

53. A releasing composition of claim 50 comprising about  
0.01 to about 5% of the cyclo-oligomer component.

20 54. A releasing composition of claim 50 comprising about  
2% of the cyclo-oligomer component, wherein the cyclo-  
oligomer component is an alpha-cyclodextrin.

25 55. A releasing composition of claim 50 comprising about  
0.05% of the cyclo-oligomer component, wherein the cyclo-  
oligomer component is a beta-randomly methylated  
cyclodextrin.

30 56. A releasing composition of claim 44 further  
comprising about 0.5 to about 5% of a metal salicylate,  
including sodium salicylate.

57. A releasing composition of claim 44 further  
35 comprising about 0.01 to about 0.1% of a surfactant.

58. A releasing composition of claim 57 wherein the surfactant is selected from the group consisting of tween-20 and triton X-100.

5 59. A releasing composition of claim 44 wherein the releasing composition forms a homogeneous mixture with a body fluid sample containing the vitamin D component.

10 60. A releasing composition of claim 59 wherein a detecting composition is added to the homogeneous mixture to determine the concentration of the vitamin D component.

15 61. A releasing composition of claim 44 being employed in a biochemical assay.

62. A releasing composition of claim 61 wherein the biochemical assay is a homogeneous biochemical assay.

20 63. A releasing composition comprising

about 0.1 to about 1.0 M of an aqueous base component;

25 about 0.01 to about 5% of a cyclo-oligomer component; and

30 about 0.01 to about 5% of a metal salicylate, the releasing composition is useful in determining the concentration of a vitamin D component.

35 64. A releasing component of claim 63 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin and the metal salicylate is sodium salicylate.



65. A releasing component of claim 63 being substantially free of an organic solvent.

66. A method of assaying a body fluid sample for the concentration of a vitamin D component, the method comprising the steps of:

releasing the vitamin D component from the vitamin D component binding-protein by contacting the sample with a releasing composition in a holder; and

determining the concentration of the vitamin D component.

67. A method of claim 66 wherein the vitamin D is released into a homogeneous mixture of the body fluid sample and the releasing composition.

68. A method of claim 66 wherein the releasing composition comprises

about 0.1 to about 1.0 M of an aqueous base component;

0 to about 5% of a cyclo-oligomer component;

0 to about 5% of a metal salicylate; and

0 to about 0.1% of a surfactant.

69. A method of claim 68 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin, the metal salicylate is sodium salicylate and the surfactant is tween-20.

70. A method of claim 66 wherein the releasing

composition is substantially free of an organic solvent,  
including an organic solvent.

71. A method of claim 67 wherein the determining step  
5 includes the steps of:

adding a detecting composition to the holder, the  
detecting composition comprises a host component and a  
partner component, the host component binds to the  
10 partner component to form a partner/host complex;

isolate the complex in the tube;

measuring the concentration of the complex by  
15 measuring the concentration of the host component in the  
complex, the concentration of the complex is proportional  
to the concentration of the vitamin D component.

72. A method of claim 71 wherein the host component is  
20 an antibody labeled with acridinium.

73. A method of claim 72 wherein the concentration of  
the host is measured by detecting the emitted level of  
chemiluminescence.

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74. A method of claim 71 wherein the partner component  
competes with a vitamin D component to form a complex  
with the host component, the partner component comprises  
a vitamin D component linked to a magnetic particle, the  
30 partner component binds to the host component through a  
vitamin D binding-protein.

75. A method of claim 66 wherein the determining step  
includes the steps of:

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adding a partner component and a vitamin D binding-

protein to the tube, the partner component competes with the vitamin D component to bind to the vitamin D component binding-protein to form a partner/binding-protein complex;

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isolate the partner/binding-protein complex in the tube; and

adding a host component, the host component binds to the partner/binding-protein complex to form a partner/binding-protein/host component complex.

measuring the concentration of the partner/binding-protein/host component complex by measuring the concentration of the host component in the complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

76. A method of claim 75 wherein the host component is an antibody labeled with acridinium.

77. A method of claim 75 wherein the concentration of the host is measured by detecting the emitted level of chemiluminescence.

78. A method of claim 75 wherein the partner component comprises a vitamin D component linked to a magnetic particle.

79. A method of claim 66 providing a more precise determination of the vitamin D component as compared to method using a releasing composition comprising an organic solvent.

80. A method of assaying a body fluid sample for the concentration of a 25-OH-D component, the method

comprising the steps of:

releasing the 25-OH-D from the 25-OH-D binding-protein by contacting the sample with a releasing  
5 composition in a holder, including a cuvette, the releasing composition comprises about 0.1 to about 1.0 M of an aqueous base component, 0 to about 5% of a cyclo-oligomer component, 0 to about 5% of a metal salicylate, and 0 to about 0.1% of a surfactant; and

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adding a detecting composition to the holder,

the detecting composition comprises an antibody labeled with acridinium, a 25-OH-D binding-protein and a partner component, the partner component  
15 comprises a 25-OH-D linked to a magnetic particle,

the partner component competes with the released 25-OH-D to bind to the 25-OH-D binding-protein  
20 to form a partner/binding-protein complex,

the antibody binds to the partner/binding-protein complex to form an antibody/binding-protein/partner component complex;  
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isolating the antibody/binding-protein/partner component complex in the tube; and

measuring the concentration of the antibody/binding-protein/partner component complex by measuring the  
30 concentration of the labeled antibody in the complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

35 81. A method of claim 80 wherein the concentration of the labeled antibody is measured by detecting the emitted

level of chemiluminescence.

82. A method of claim 80 wherein the releasing composition comprises about 0.35 to about 0.5 M of NaOH,  
5 about 2% of alpha-cyclodextrin and about 2% of salicylate.

83. A method of claim 80 wherein the releasing composition is substantially free of an organic solvent,  
10 including an organic solvent.

84. A method of assaying a body fluid sample for the concentration of a vitamin D component, the method comprising the steps of:

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forming a homogeneous mixture of a body fluid sample containing the vitamin D component and a releasing component; and

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adding a detecting composition to the mixture to determine the concentration of the vitamin D component.